Charting a Course for our Profession (and Industry): Food Animals

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So where to from here

- 1. Veterinarians should have control of all uses of antimicrobials in animals.
- 2. Emphasize veterinary education on optimal use of these resources.
- 3. Duration of therapy research is an absolute requirement
- 4. Continue the emphasis on prevention of infectious disease

So where to from here

- 5. Revisit efficacy research for many of the preventive applications (especially administered to a group through feed and water) to see if we actually still make a difference. (Who's going to pay?)
- 6. Enforce our current regulations!!
- 7. Include data and the correct analysis in the decision process
- 8. It is reasonable to monitor both antibiotic resistance and antibiotic use

Classes of Antimicrobial Use in Food Animals

- We get confused as to the reason for classification
  - Therapeutic intent?
  - Probability of selection for resistant bacteria?
  - Societal justification?

Classes of Antimicrobial Use in Food Animals

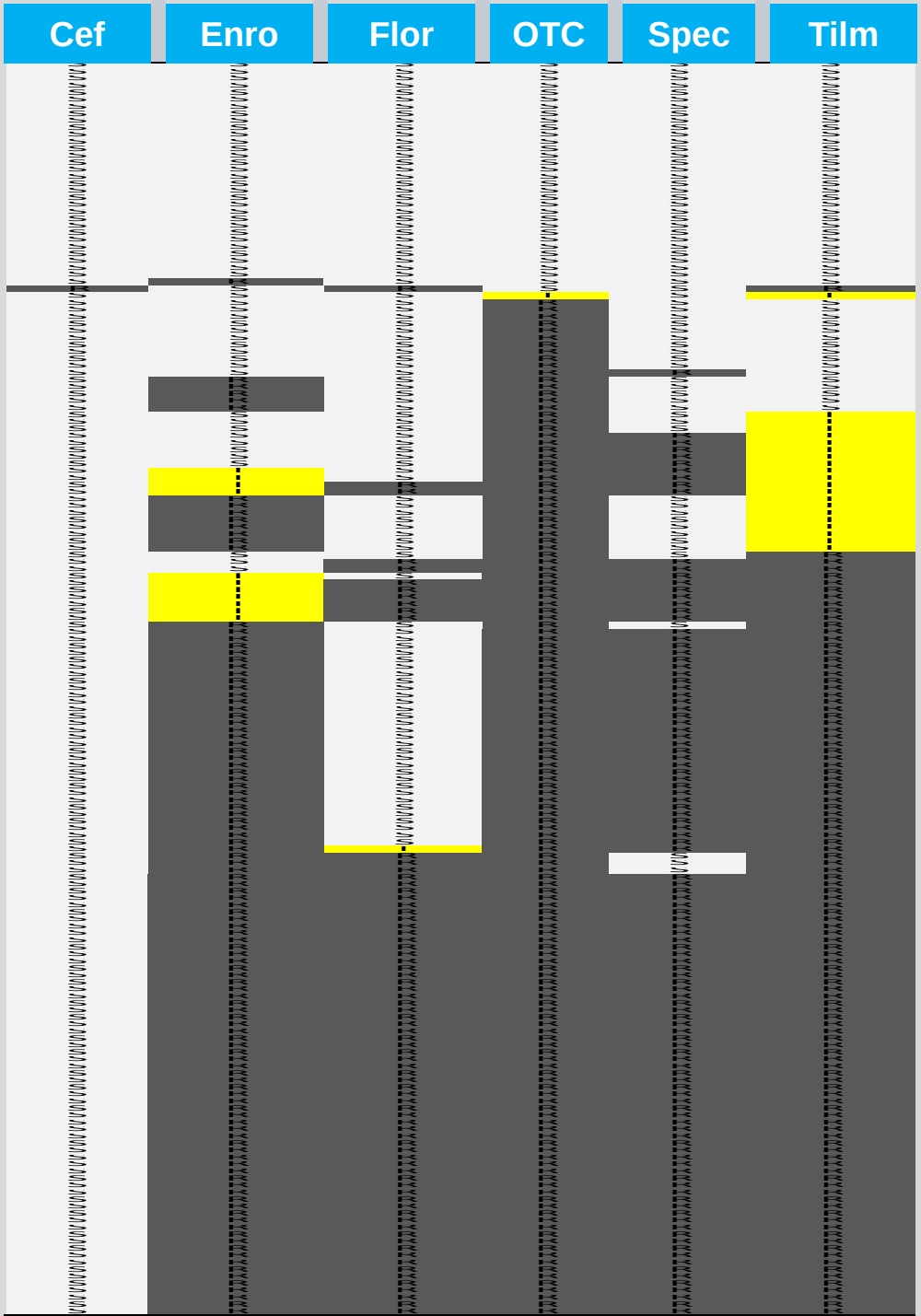
- FDA/CVM approval classifications
  - ~~Increase in rate of gain~~
  - ~~Increase in feed efficiency~~
  - Prevention
  - Control
  - Therapy/Treatment
- Classifications by bacteria
  - They don't care

2011 KSU *M. haem* isolates N = 179

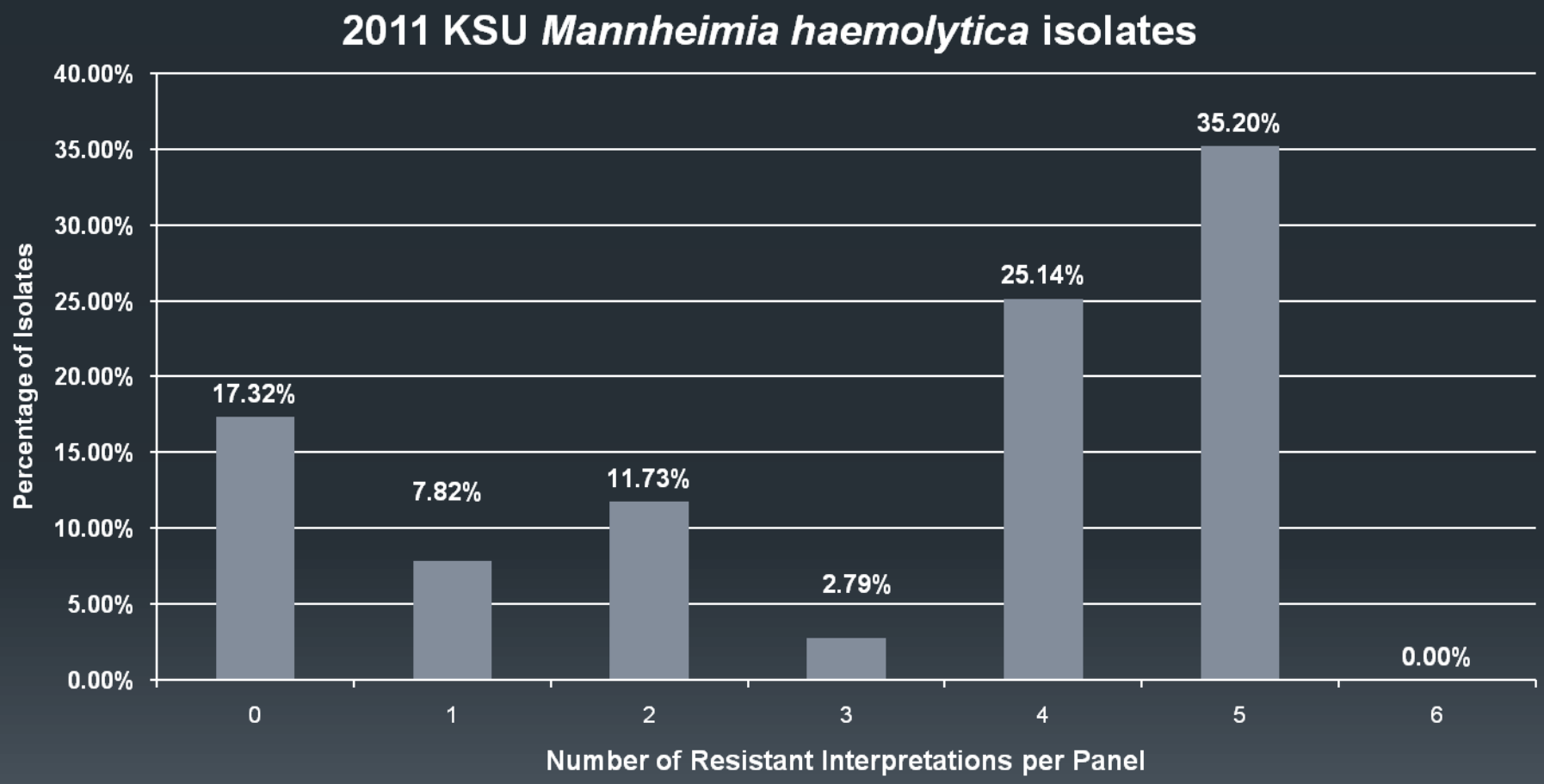
Unshaded = susceptible

Yellow = intermediate

Gray = resistant



153 (85%) were direct matches for tilmicosin and tulathromycin, others were:
(14) Tul S, Til I,
(7) Tul R, Til I,
(3) Tul I, Til R


2011 KSU *Mannheimia haemolytica* isolates
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Percentage of Isolates
17.32%
7.82%
11.73%
2.79%
25.14%
35.20%
0.00%
0
1
2
3
4
5
6
Number of Resistant Interpretations per Panel

The Regulatory Process Matters

- Cephalosporin ELDU prohibition
  - Initially to be all extralabel uses in all food animal species (2008)
  - Withdrawn based on approximately 300 comments
  - Came back in 2012 as
    - Specific ELDU prohibited in major food animal species
      - ELDU in minor species OK
    - Extralabel applications OK, but not extralabel regimens
    - ELDU for control OK, but not for prevention
      - The problem is that CVM cannot provide definitions discerning between the two to guide us.

Cephalosporin ELDU

- The final rule contained…
  - Misinterpretation of key references in relation to justification for removal of ELDU in cattle
  - Selective omission of readily available articles which supported an alternative view
  - No justification for removal of ELDU in swine
  - No evidence to support the contention that resistance changes of concern were related to extralabel use as opposed to label use of the drug

In response to assertions that the FDA is using the precautionary principle

"However, the Agency believes that it is not limited to making risk determinations based solely on documented scientific information, but may use other suitable information as appropriate."

USING TETRACYCLINES AS AN EXAMPLE

But first, basic math

$$\% = \frac{X}{Y}$$

Or…

$$\% = \frac{X}{Y}$$

The Tetracyclines - Pharmacodynamics

- Trying to predict - Time above MIC? Or AUC/MIC?
  - There is only one paper that I have found which addresses the first generation tetracyclines (CTC, OTC, TC).
    - An E-max model for tetracycline displayed bacteriostatic activity against *E. coli*. (Regoes, 2004)
  - Information on AUC/MIC, T>MIC, or Cmax:MIC is not available in the literature for the first generation tetracyclines.
  - These data are often for different organisms in culture, anyway.

The Tetracyclines - Pharmacodynamics

- Regardless of what we predict as to pharmacodynamic indices for the tetracyclines, they may or may not apply to gut activity anyway.
- Even for systemic effects, treating pharmacodynamic indices as absolutes will likely lead us astray.
  - i.e., what happens below the MIC?
  - Where is the concentration measured?

The Tetracyclines – "S", "I", and "R"

- What is "resistant"?
- Classic veterinary breakpoints adapted from human medicine are 4, 8, and 16 µg/ml for "S", "I", and "R", respectively.
- These are substitution variables for *in vivo* activity based on the ability of the antimicrobial to inhibit growth in the laboratory.
- There are now "generic" breakpoints for swine and bovine respiratory disease.

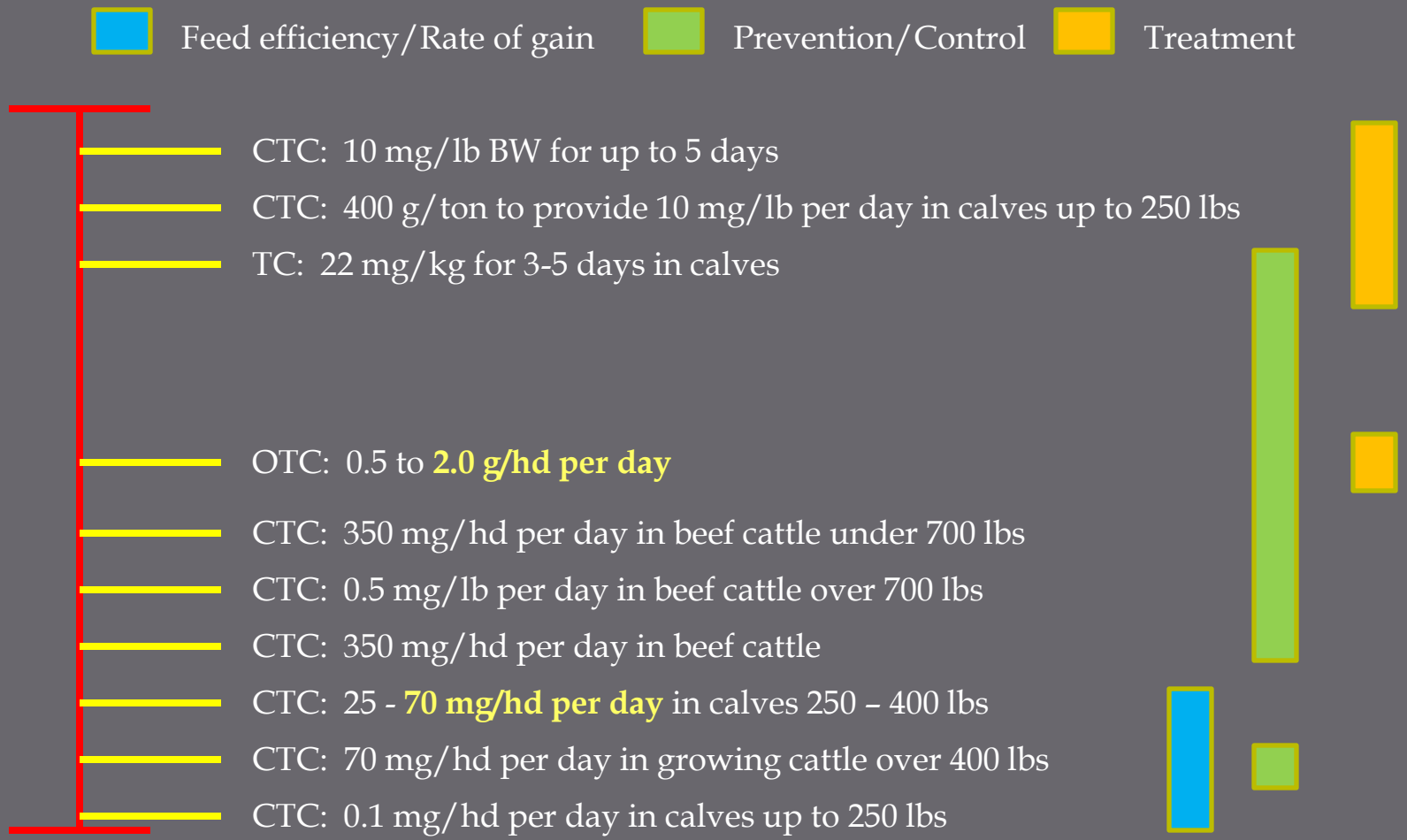
The Tetracyclines – Resistance Genetics

- There are extensive, transmissible resistance genetic elements out there
  - e.g., a 2010 review of the tetracycline resistome notes 1,189 different reported resistance genes present in 84 bacterial genera, which included 354 bacterial species (Thaker, 2010)
  - These genes comprise 41 classes, with three major mechanisms
    - Actively pumping the drug out of the cell
    - Enzymatic degradation of the drug
    - Protection of the drug binding site

The Tetracyclines – Resistance Transfer

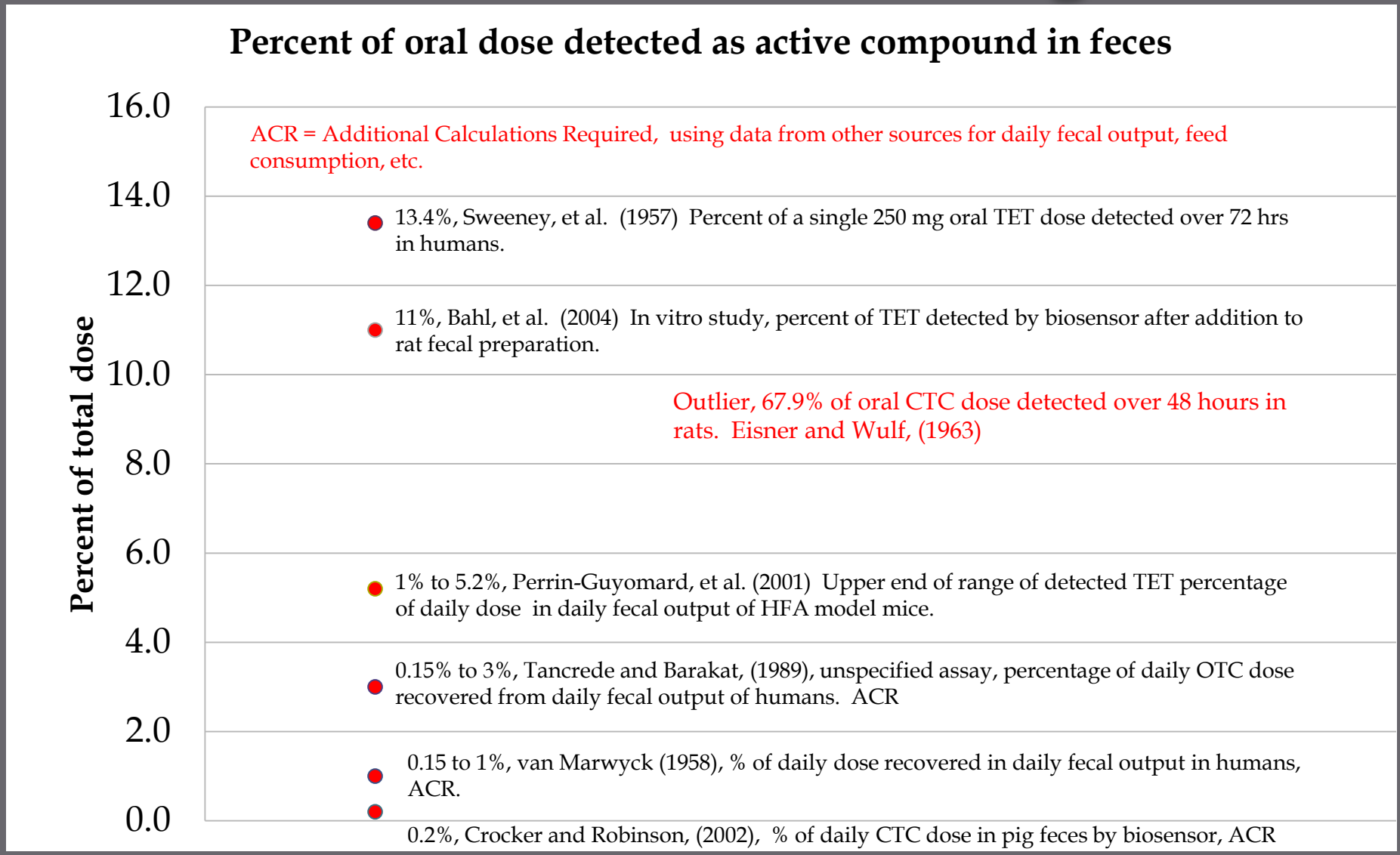
- Chopra and Roberts (2001)
  - Gram-negative and Gram-positive genes coding for tetracycline efflux are generally associated with plasmids.
  - tet(S) and tet(O) encode for ribosomal protection and are located both in the chromosome and in conjugative plasmids
  - tet(M) and tet(Q) (also ribosomal protection) and typically associated with conjugative transposons
  - Other mechanisms include enzymatic inactiviation (tet(X) and tet(37)
  - Mosaic genes have also been described, which are combinations of individual genes (e.g., tet(O/32/O)

U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)



These are not all of the CTC, TC, and OTC indications, but are selected to illustrate the regimen range.

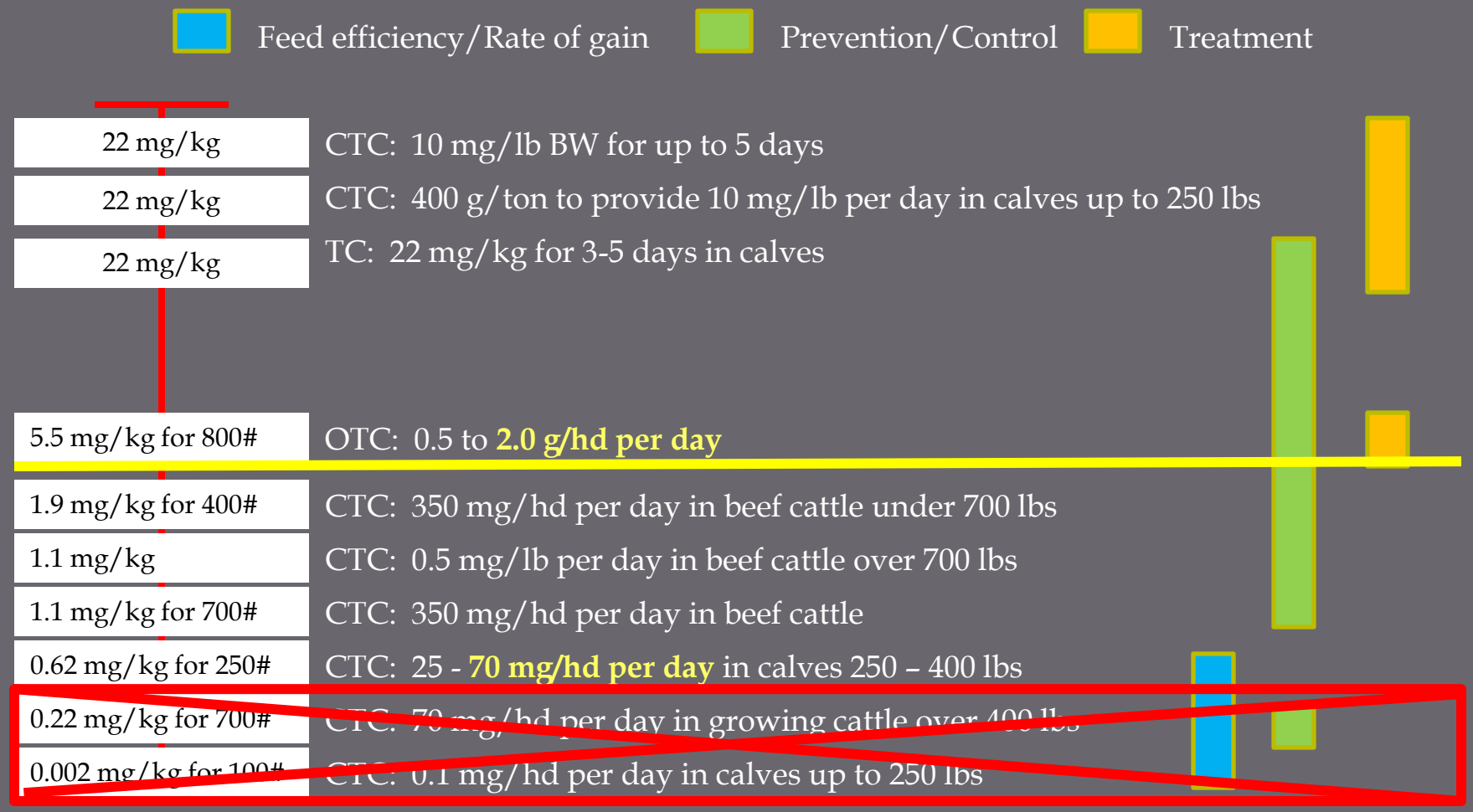
By the way, how much tetracycline remains active in the gut?



NOAEL for Tetracycline?

- Carmen, et al. (2006) evaluated three concentrations of tetracycline in a chemostat system inoculated with human fecal flora.
- Concentrations of 0.15, 1.5, and 15 µg/ml were used in the systems, equivalent to daily doses of 0.025, 0.25, and 2.5 mg/kg per day in a 60 kg human (based on fecal concentration data by van Marwyck, 1958).
- Statistical analysis identified the lowest and middle concentrations as having no observable adverse effect on the bacterial population.

U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)



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NOAEL for Tetracycline?

- Perrin-Guyomard, et al. (2001) used a human-flora-associated (HFA) mouse model to evaluate water tetracycline concentrations of 0, 1, 10, and 100 mg/liter administered for 8 weeks.
- Upon further calculation, these are equivalent to doses of 0, 0.125, 1.25, and 12.5 mg/kg BW.
- The authors cited the highest dose as being capable of disrupting the capability to resist Salmonella infection by a resistant isolate.
- At the lowest dose, there were transient increases in percent resistant *Bacteroides fragilis* and Enterococci. These effects were more pronounced at higher doses.

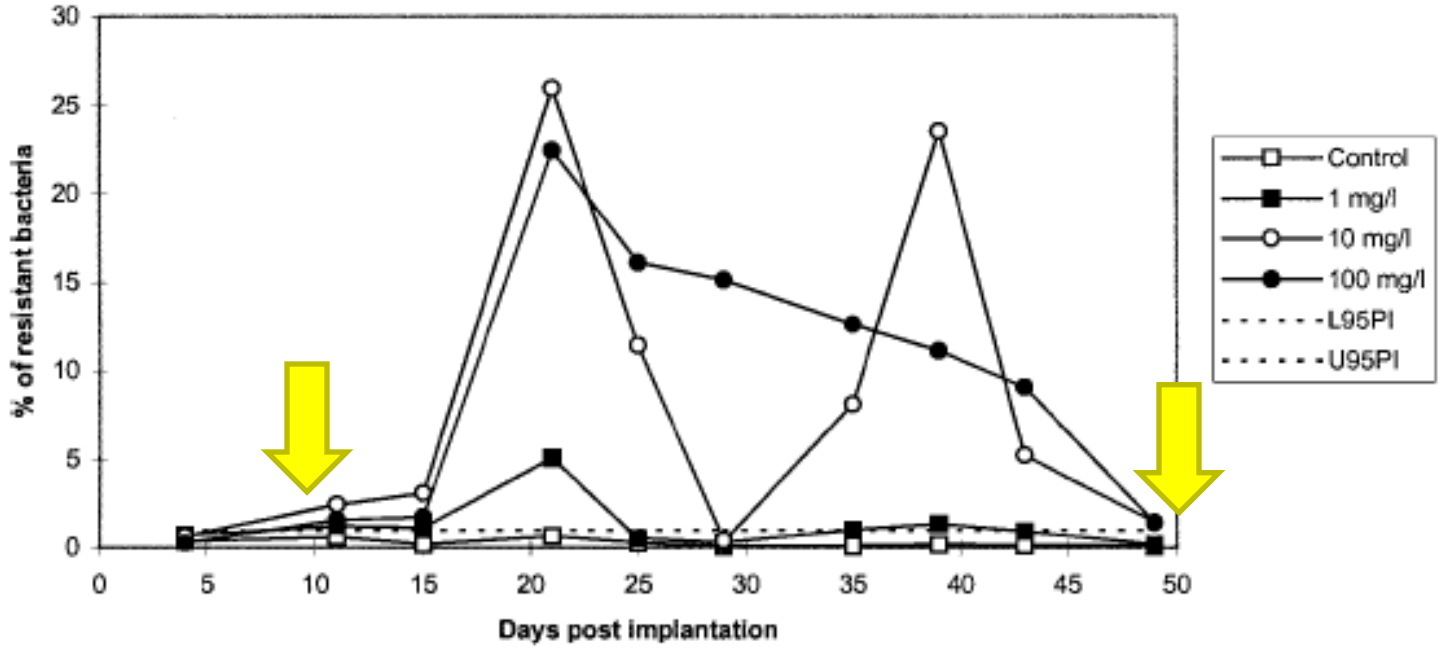
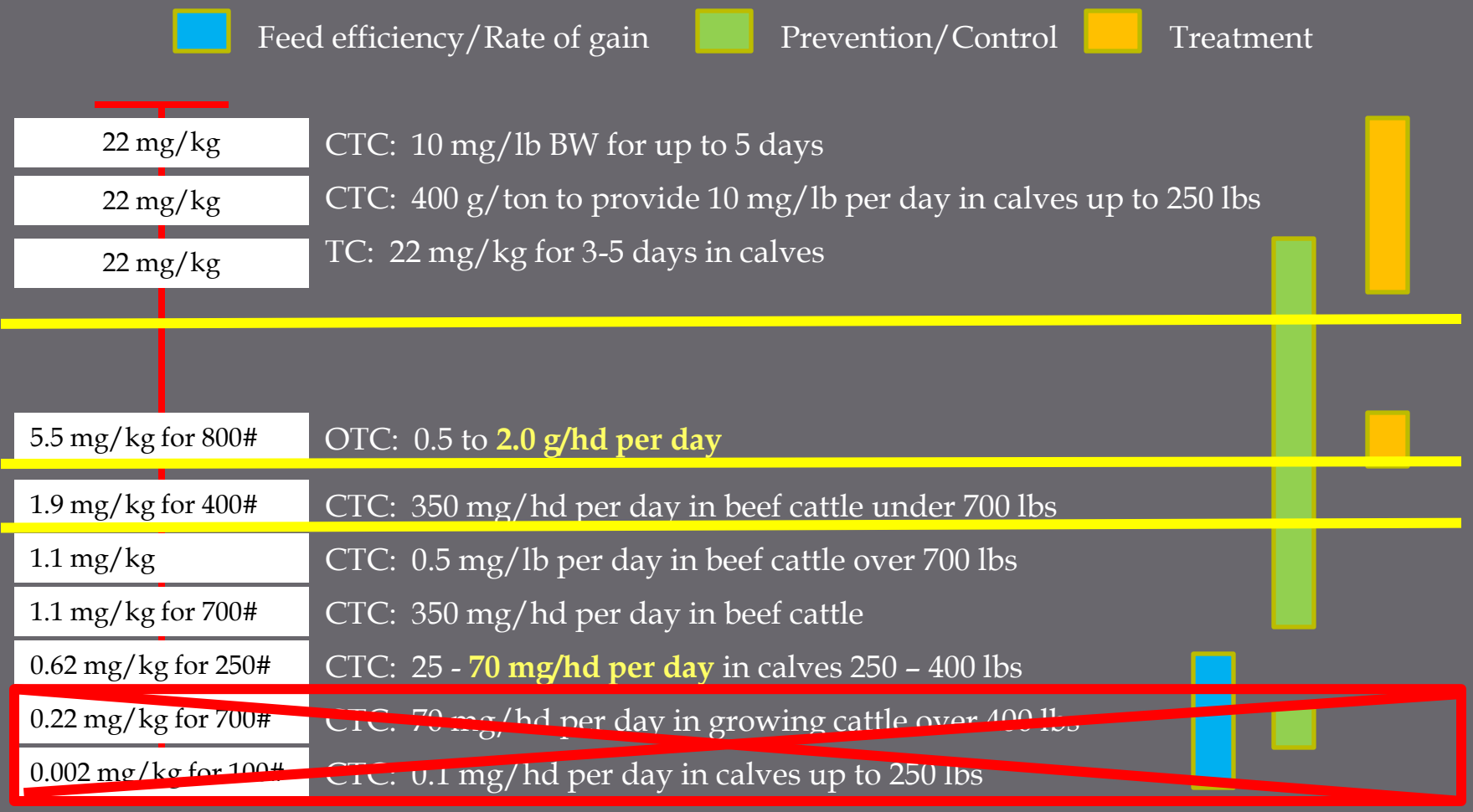


FIG. 2. Time course evolution of the levels of resistant *Bacteroides fragilis* in colonic bacteria of male HFA mice during tetracycline exposure (second trial). Treatment period lasted from d.p.i. 10 to 50. L95PI and U95PI indicate respectively the lower 95% prediction interval and the upper 95% prediction interval, estimated from the control values.

Perrin-Guyomard, et al. (2001)

In the first trial, the background of Gram-negative anaerobes and *B. fragilis* resistant to tetracycline (32 μg/ml) was very low at the start of treatment and remained lower than 2% in control mice during the treatment period. Tetracycline at 10 and 100 mg/liter significantly increased the percentage of resistant Gram-negative anaerobes in female mice (3 and 8%, respectively) and the antibiotic at 100 mg/liter significantly increased the levels of resistant Gram-negative anaerobes in males (9.5%). The percentage of resistant *B. fragilis* significantly increased (25% in males and 15% in females) with 100 mg/liter of tetracycline in both genders. In female mice, the percentage of resistant Gram-negative anaerobes in the animals previously treated with 100 mg/liter remained above the control group during the posttreatment period (data not shown).

U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)



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NOAEL for Tetracycline?

- Tancrede and Baraket (1987) administered 2, 20, or 2000 mg/day to human volunteers for 7 days.
- In 60 kg humans, this would be equivalent to 0.03, 0.33, 33 mg/kg per day.
- The low dose caused no change in % resistance in the dominant anaerobes.
- The two high doses did induce changes in resistance.

U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)


Feed efficiency/Rate of gain


Prevention/Control


Treatment

22 mg/kg	CTC: 10 mg/lb BW for up to 5 days
22 mg/kg	CTC: 400 g/ton to provide 10 mg/lb per day in calves up to 250 lbs
22 mg/kg	TC: 22 mg/kg for 3-5 days in calves
5.5 mg/kg for 800#	OTC: 0.5 to **2.0 g/hd per day**
1.9 mg/kg for 400#	CTC: 350 mg/hd per day in beef cattle under 700 lbs
1.1 mg/kg	CTC: 0.5 mg/lb per day in beef cattle over 700 lbs
1.1 mg/kg for 700#	CTC: 350 mg/hd per day in beef cattle
0.62 mg/kg for 250#	CTC: 25 - **70 mg/hd per day** in calves 250 – 400 lbs
0.22 mg/kg for 700#	CTC: 70 mg/hd per day in growing cattle over 400 lbs
0.002 mg/kg for 100#	CTC: 0.1 mg/hd per day in calves up to 250 lbs

These are not all of the CTC, TC, and OTC indications, but are selected to illustrate the regimen range.

Caveats

- All models are wrong, some are just useful
- These studies are not presented as predicting NOAELs in food animals, however…
- they do display a consistent dose-effect relationship, with higher doses having a greater effect on fecal flora during the same dosing interval.
- Changes from the lower doses were often shown to be transient, even for prolonged administration.

TETRACYCLINES – WHAT ACTUALLY HAPPENS?

CTC in Feed

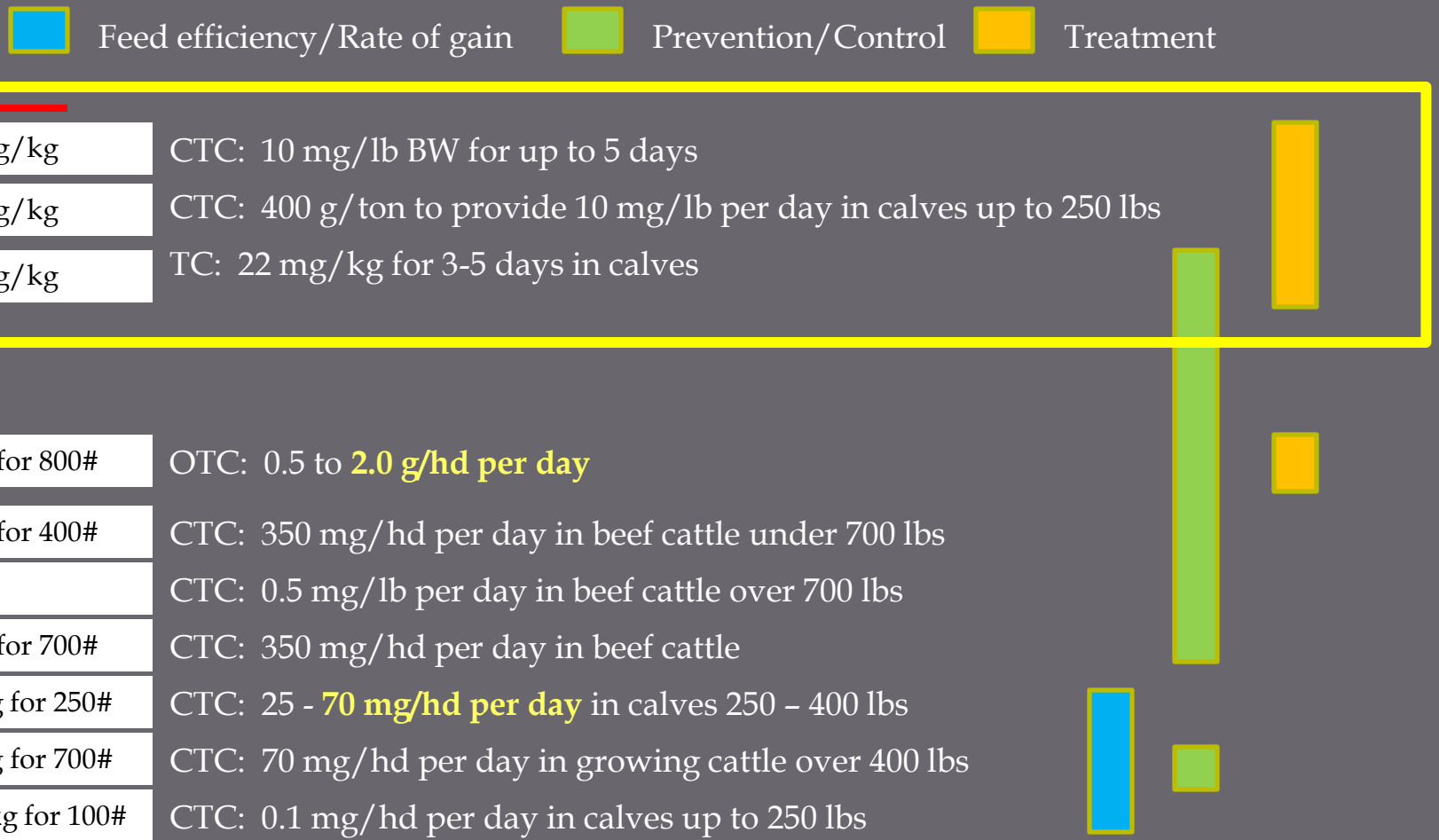
- CTC at 22 mg/kg BW in feed for days 0 through 4, 6 through 10 and 12 through 16.
- Fecal samples on days -7, 0, 2, 6, 8, 12, 14, 19, 22, 26, and 33.
- Resistance to CTC in *E. coli* and *Enterococcus* was monitored.
- Exposure to CTC was associated with a significant temporary increase in log2 MIC for both genera, but returned to pre-exposure values by day 33.

CTC in Feed

- All ceftiofur resistant E. coli isolates were also resistant to tetracycline, but…
- Exposure to chlortetracycline led to a significant decrease in the proportion of E. coli resistant to ceftiofur during exposure.

Platt TM, Loneragan GH, Scott HM, et al. Antimicrobial susceptibility of enteric bacteria recovered from feedlot cattle administered chlortetracycline in feed. Am J Vet Res 2008;69, 988-996.

U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)



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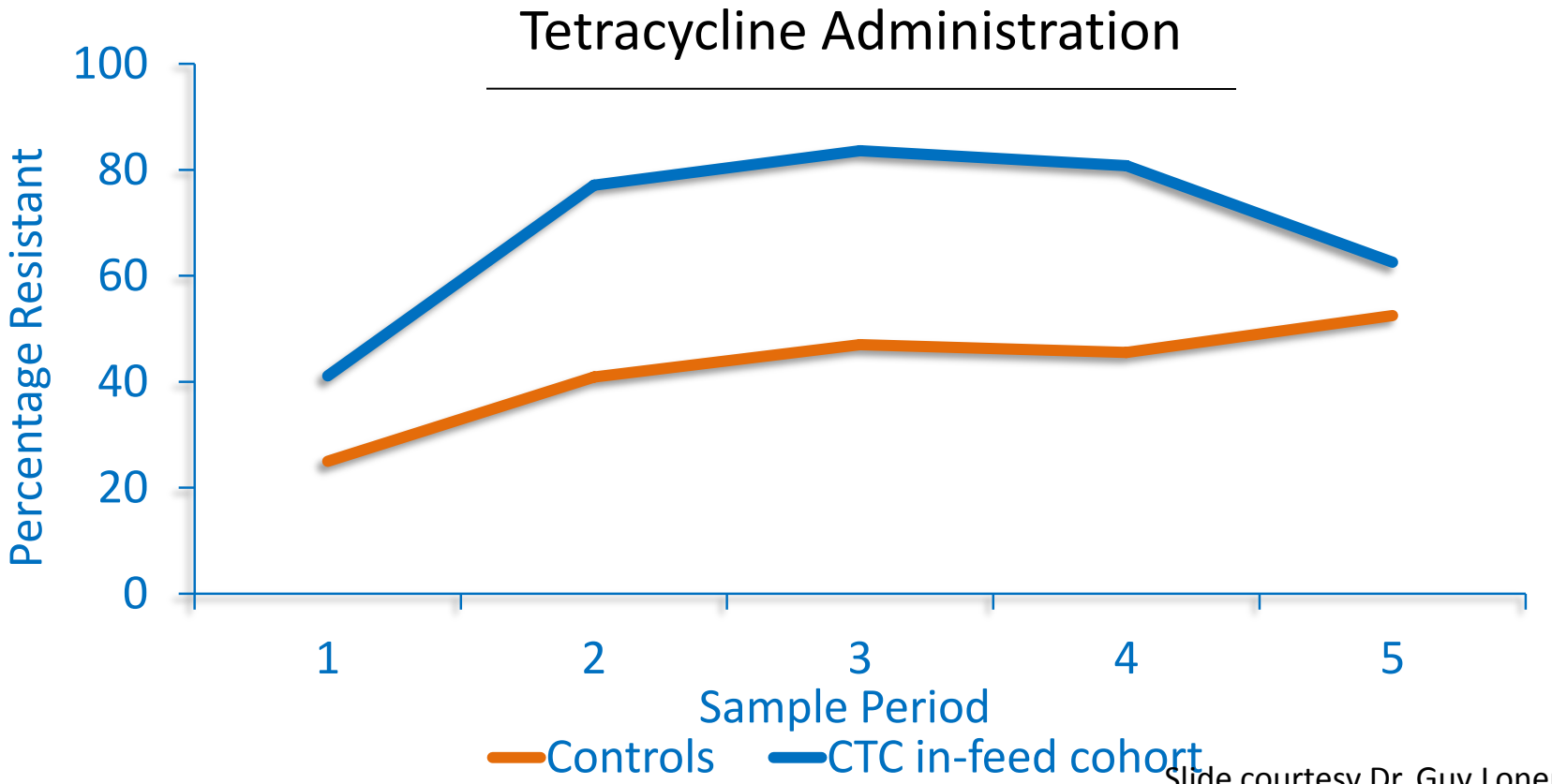
Tetracycline Resistance

- A function of concentration and time
  - Goes to baseline when drug cleared from system
  - Regardless of whether animal, human, or *in vitro*



Slide courtesy Dr. Guy Loneragan

"Subtherapeutics" and E. coli

- 300 crossbred steers on 6 treatments. (5 pens of 10 each treatment). Label inclusion rates.
  - Control
  - Chlortetracycline/sulfamethazine 44 ppm each (Aureo S-700)
  - Chlortetracycline 11 ppm (Aureomycin)
  - Monensin 25 ppm (Rumensin)
  - Tylosin 11 ppm (Tylan)
  - Virginiamycin 31 ppm (V-Max)

“Subtherapeutics” and E. coli

- Silage-based diet for first 115 days, adapted to a barley-based diet over 21 days and then fed for an additional 179 days.
- The treatments were administered starting at 17 days and included for 61 days in the silage diet, then discontinued for 86 days.
- The treatments were reintroduced for a period of 42 days during the grain based diet.

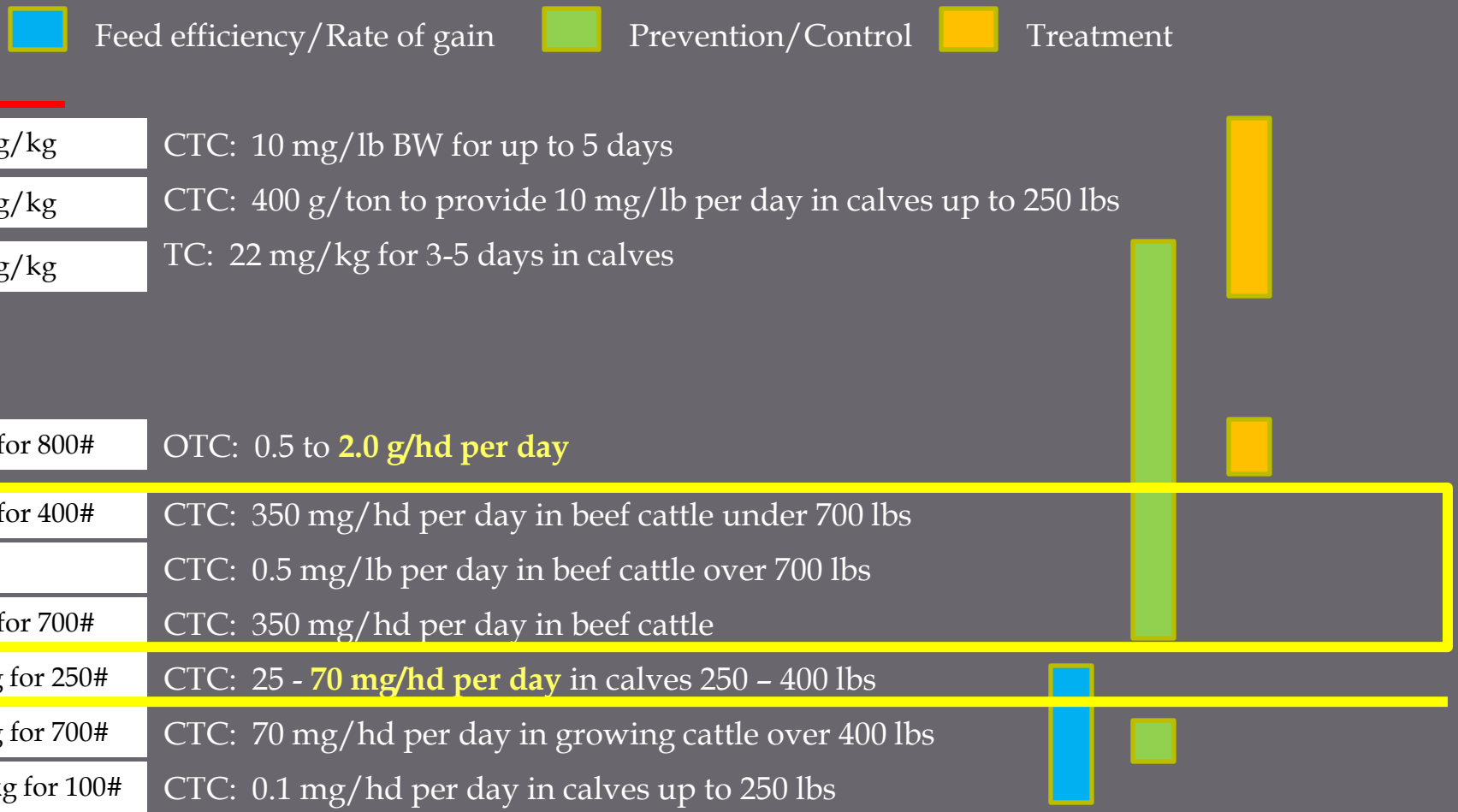
“Subtherapeutics” and E. coli

- In-weights of 198 ± 20 kg
- Figure a 1 kg/day gain during the 115 day backgrounding period (end weight 313 kg, average weight for period of **255 kg**)
- For the feeding period, figure a 1.6 kg/day gain for the 200 day period, for a final weight of 575 kg (1265 lbs). The medicated feed was fed from days 51 to 93 of the finishing period, for an estimated average weight during the administration period of **428 kg**.

"Subtherapeutics" and E. coli

- Cattle were consuming about 7.8 kg/day (DMB) during backgrounding period (silage) then about 11.0 kg/day during finishing.
- Antimicrobial Intake would therefore be…
  - Backgrounding period (administered 61 days)
    - Chlortetracycline/sulfamethazine – 343 mg (1.4 mg/kg) each compound/day
    - Chlortetracycline – 85.8 mg (0.3 mg/kg)/day
  - Feeding period (administered 42 days)
    - Chlortetracycline/sulfamethazine – 484 mg (1.1 mg/kg) each compound/day
    - Chlortetracycline – 121 mg (0.3 mg/kg)/day

U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)



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"Subtherapeutics" and E. coli

- Except for control and monensin groups, the number of E. coli isolated on non-selective media were lower in the silage period.
- Including tetracycline alone in the diet increased the tetracycline-resistant E. coli population from approximately 3% of isolates to 10%.
- Tetracycline/sulfamethazine increased the percentage to 19.5%.
  - And also increased the percentage of ampicillin-resistant E. coli isolates.

"Subtherapeutics" and E. coli

- Removing the treatments from the diets for 56 days during the silage period and 40 days during the grain period did not significantly alter the prevalence of cattle shedding tetracycline- or ampicillin-resistant E. coli.

Alexander TW, et al. Effect of subtherapeutic administration of antibiotics on prevalence of antibiotic-resistant Escherichia coli bacteria in feedlot cattle. Applied and Environmental Microbiology 2008;74,4405-4416.

Diversity and Distribution of E. coli administered "subtherapeutics"

- 197 day study administering either CTC (350 mg/hd per day) or CTC/sulfamethazine (same rate each per day)
- "…E. coli from day 0 showed diverse antibiogram profiles and strain types, which by the finishing phase were limited to up to three, irrespective of the treatment."
- "…an increased linked inheritance of ampicillin and tetracycline resistance genes and prevalence of specific strains at day 197."

Diversity and Distribution of E. coli administered "subtherapeutics"

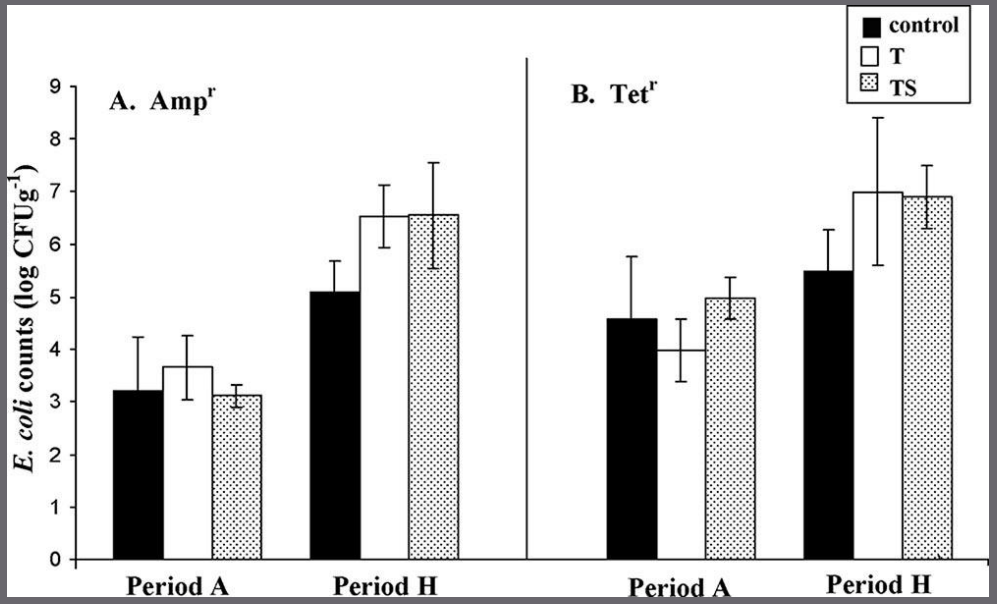

FIG. 2. Ampr (A) and Tetr (B) *E. coli counts (log CFU g1 [wet* weight]) in periods A and H with no antibiotic treatment (control), 350 mg head/ day chlortetracycline (T), and 350 mg head/day each chlortetracycline and sulfamethazine (TS).

Sharma R, et al. Diversity and distribution of commensal fecal *Escherichia coli* bacteria in beef cattle administered selected subtherapeutic antimicrobials in a feedlot setting. Applied and Environmental Microbiology 2008;74,6178-6186.

Take-Home Message on *in vivo* Antimicrobial Gut Activity

- Very complicated, but we do cause changes in enteric populations with oral antimicrobial use
- A definite dose-response relationship demonstrated in some studies.
- In some studies, the changes were transient in at least some of the categories.
- If we lop off the most politically acceptable category to "cut down use", then we end up with a precedent of the precautionary principle for addressing the much more important, and in my mind the more likely to have an effect, prevention and control claims.

Let's not become fixated on the red light!

- We also have developing issues of resistance in certain classes of food animal pathogens.
  - *Salmonella newport*
  - *Mannheimia haemolytica*
  - *Pasteurella multocida*

In my opinion…

- The example of the tetracyclines illustrates the multifaceted interaction between antimicrobials and enteric organisms as well as food animal pathogens.
- In relation to antimcrobial resistance regulation and legislation, antimicrobial use classification as “subtherapeutic” or “therapeutic” across all antimicrobials is about societal justification, not about potential for resistance selection in enteric bacteria populations.

In my opinion…

- The relative resistance selection contribution of dose and duration is ill-defined
  - In fact, the effect of duration of **therapy** on therapeutic outcome is ill-defined in both human and veterinary medicine
  - In food animal medicine, we have multiple studies on post-treatment intervals after single injections, but very little on the effects of duration of therapy.